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Epidemiological Interpretation of Studies Examining the Effect of Antibiotic Usage on Resistance

Vered Schechner,[a] Elizabeth Temkin,[a,b] Stephan Harbarth,[c] Yehuda Carmeli,[a,b] Mitchell J. Schwaber[b]

Division of Epidemiology and Preventive Medicine, Tel Aviv Sourasky Medical Center, Tel-Aviv, Israel[a]; National Center for Infection Control, Israel Ministry of Health, Tel Aviv, Israel[b]; Division of Infectious Diseases and Infection Control Program, University of Geneva Hospitals and Medical School, Geneva, Switzerland[c]

SUMMARY

Bacterial resistance to antibiotics is a growing clinical problem and public health threat. Antibiotic use is a known risk factor for the emergence of antibiotic resistance, but demonstrating the causal link between antibiotic use and resistance is challenging. This review describes different study designs for assessing the association between antibiotic use and resistance and discusses strengths and limitations of each. Approaches to measuring antibiotic use and antibiotic resistance are presented. Important methodological issues such as confounding, establishing temporality, and control group selection are examined.

INTRODUCTION

Bacterial resistance to antibiotics is an increasing clinical problem worldwide and a significant public health threat (1). Selective antibiotic pressure is an important determinant of emergence and dissemination of antibiotic resistance (2). Moreover, it is among the few modifiable factors predisposing to antibiotic resistance. Studies evaluating the association between antibiotic exposure and antibiotic resistance are subject to common pitfalls, including inadequate adjustment for important confounding variables, control group selection, extent of prior antibiotic exposure, and measurements of resistance outcomes. In addition, different study designs; units of analysis, i.e., the group or the individual; and approaches to measuring exposure and outcome have been used to evaluate the association between antibiotic exposure and antibiotic resistance. The heterogeneity of studies makes them difficult to compare (3). The goals of the present article are to describe different methodological approaches to studying the impact of antibiotic use on resistance and to point out strengths and limitations of each approach in order to help readers become critical reviewers of this body of research. While resistance appears in a wide variety of pathogens in different settings, for reasons of space, we will not discuss in detail studies addressing antibiotic resistance in the environment, livestock, and the community setting, but certain analogies may be drawn from the ideas and lessons presented here.

HISTORICAL PERSPECTIVE

Studies of the association between antibiotic use and resistance are nearly as old as antibiotics themselves. These early studies were in the form of descriptive reports or case series. The first class of antibiotics, sulfonamides, became widely available in the late 1930s. By 1940, a North Carolina physician reported the declining success of sulfanilamide in the treatment of gonorrhea (4). He noted that in contrast to the 75 to 90% cure rate described in the medical literature in 1937 to 1938, only 2 of the 15 previous cases seen in his clinic had responded to treatment. He offered a clear explanation of selection pressure (without using that term) (4):

Address correspondence to Mitchell J. Schwaber, mitchells@tlvmc.gov.il.

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TABLE 1 Comparison of measures for reporting antibiotic use[a]

Measure of antibiotic use	Calculation	Advantages	Disadvantages
DDD (total grams of a specific antibiotic per unit time divided by the WHO reference value) (the reference value is the avg maintenance dose per day for a drug used for its main indication in adults)	WHO reference values, oral ciprofloxacin = 1 g, i.v. ceftriaxone = 2 g, i.v. azithromycin = 0.5 g; for oral ciprofloxacin use, 28 g/1 g = 28 DDDs; for i.v. ceftriaxone use, 15 g/2 g = 7.5 DDDs; for i.v. azithromycin, 7.5 g/0.5 g = 15 DDDs; total antibiotic use this month, 50.5 DDDs per 100 patient-days	Easy to collect (no patient-level data needed); allows standardized comparisons between drugs (regardless of route) and between settings	Creates biased estimations when the true administered dose differs significantly from the reference value, e.g., in children; WHO revises reference values from time to time, making longitudinal comparisons difficult; some WHO reference values differ from the typically prescribed dose
DOT (sum of days in which each antibacterial drug is administered)	28 days of ciprofloxacin + 15 days of ceftriaxone + 15 days of azithromycin = 58 DOTs per 100 patient-days	Accurate estimation of polydrug therapy; does not matter whether a nonstandard dose was given (e.g., accurate for children)	Patient-level data are needed; no reflection of the dosage given
LOT (no. of days that a patient receives an antimicrobial drug irrespective of the no. of different drugs)	28 days of ciprofloxacin + 15 days of both ceftriaxone and azithromycin = 43 LOTs per 100 patient-days	Accurate estimation of duration of therapy; does not matter whether a nonstandard dose was given (e.g., accurate for children)	Patient-level data are needed; no reflection of polydrug therapy; no reflection of the dosage given; cannot be used to compare usage of individual drugs

[a] Example used in calculations: in ward A over 1 month, 4 patients received 1 g/day ciprofloxacin orally for 7 days (ward total = 28 g), and 3 patients received 1 g/day ceftriaxone intravenously (i.v.) for 5 days (ward total = 15 g) plus 0.5 g/day azithromycin i.v. for 5 days (ward total = 7.5 g). Ward occupancy during this month was 100 patient-days. DDD, defined daily dose; DOT, days of therapy; LOT, length of therapy.

> Assuming that certain strains of gonococci are resistant to sulfalinamide, eventually all sulfalinamide-susceptible gonococci will have been killed and all gonorrhea will be caused by strains of supergonococci against which sulfalinamide will be useless! Indeed, this is almost true today.

In 1942, 2 years before the mass production of penicillin, researchers in Massachusetts published a case series of patients infected with *Staphylococcus aureus* and treated with penicillin (5). Four patients from whom serial cultures were taken throughout their treatment demonstrated declining susceptibility to the drug. For three of them, cultures taken before the start of therapy were compared to later isolates and found to be much less resistant. Those authors concluded that, "in certain subjects penicillin therapy may result in the development of resistant strains" (5). In 1947, a British physician documented an increase in the proportion of *Staphylococcus pyogenes* isolates resistant to penicillin (6). In her hospital, over 2 observation periods in 1946 and 1947, all *Staphylococcus pyogenes* isolates cultured from lesions were tested for penicillin susceptibility: in less than a year, the proportion of resistant isolates rose from 14% to 38%. The researcher attributed the change to rising antibiotic use and predicted dire consequences (6):

> In any hospital using large quantities of penicillin (and what hospital is not nowadays?) bacteria resistant to its action are probably increasing at the expense of those that are sensitive, and it seems not impossible that in time the resistant organisms will be the sole survivors.

These early observations foreshadowed a predictable pattern in which a new class of antibiotics was introduced and reports of resistance soon followed. Over time, more sophisticated research methods have been used to measure antibiotic use and resistance and to examine the association between them. What follows is a critical review of these methods.

MEASURING EXPOSURE: ANTIBIOTIC USE

Several indicators have been used to measure antibiotic use at the group level; the question of which is best remains unresolved (Table 1). The most commonly used metric is the defined daily dose (DDD), as proposed by the World Health Organization (WHO), generally expressed as DDDs per 100,000 population (for outpatient use) and DDDs per 1,000 patient-days (for inpatient use) (7). This measure allows standardized comparisons between institutions or countries (or within an institution over time or between departments), and the data needed for calculation are easily available. However, there are several limitations to DDDs: this metric will over- or underestimate true antibiotic consumption if the administered daily dose differs significantly from the WHO-defined DDD, DDDs have not been determined for children or patients with renal failure, and the WHO occasionally updates DDDs, which complicates comparisons over time (8–10). Alternative measures for antibiotic consumption are days of therapy (DOT) for each antibiotic administered (e.g., 3 different antibiotics taken for 3 days each equals 9 DOTs) and length of therapy (LOT), also known as antimicrobial exposure time, which is the number of days in which a patient receives an antibiotic irrespective of the number of different drugs (e.g., 3 different antibiotics taken for 3 days each equals 3 LOTs) (11). While LOT gives a more accurate estimation of the duration of therapy, neither LOT nor DOT reflects the dosage given, and both require individual-level data. As with DDD, DOT and LOT can be expressed as a density, i.e., DOT (or LOT) per 1,000 patient-days (12, 13). A limitation of assessing antibiotic exposure is that all these measurements (DDD, DOT, and LOT) reflect the amount of drug prescribed; the amount taken by the patient may be less if compliance is imperfect, a problem that is relevant to studies conducted in outpatient settings.

In addition to quantity, how other characteristics of antibiotic exposure are defined may influence results. First, prior antibiotic

use can be described as a categorical variable (exposed or unexposed) or as a continuous variable (number of days of treatment). Hyle et al. evaluated risk factors for extended-spectrum-beta-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella* spp. by separately analyzing studies based on measurement of prior antibiotic use as a categorical versus a continuous variable (14). They found that the use of third-generation cephalosporins was a risk factor for infection with ESBL-producing *E. coli* and *Klebsiella* spp. when antibiotic use was described as a continuous variable but not when antibiotic use was described as a categorical variable. Carmeli et al. examined antecedent treatments with different antibiotics as risk factors for vancomycin-resistant *Enterococcus* (VRE) infection (15). They found some antibiotics to be risk factors when measured as a dichotomous variable and others to be risk factors when measured continuously by duration of therapy, which may suggest that different drug classes have different modes of selection for antibiotic resistance. Second, if antibiotic use is treated as a categorical variable, a minimum length of therapy should be defined. Hyle et al. (16) studied risk factors for fluoroquinolone-resistant *Pseudomonas aeruginosa*. They constructed four models, each with a different definition of antibiotic exposure: (i) any use, (ii) >24 h of use, (iii) >48 h, and (iv) >72 h. In all models, prior fluoroquinolone use was an independent risk factor for fluoroquinolone-resistant *P. aeruginosa*; however, the strength of the association increased as the duration of use increased. Third, researchers must determine the time frame for exposure. Lipsitch reviewed studies of the association between antibiotic use and penicillin-resistant *Streptococcus pneumoniae* infection (17). The period of exposure varied from use in the past 6 months (at most) to current use at the time of *S. pneumoniae* infection (at least). In general, associations were weaker in the studies that defined exposure with a wider time frame. Fourth, the exposure can be classified at the level of drug (e.g., ciprofloxacin), class (e.g., fluoroquinolones), or spectrum of activity (e.g., antipseudomonals). In two studies, different risk factors emerged as significant depending on whether antibiotic exposure was classified at the class level or at the spectrum level (18, 19).

MEASURING OUTCOME: ANTIBIOTIC RESISTANCE

Studies may define their outcome as (i) the presence or absence of resistance to a given antibiotic, where the threshold for resistance can either include or exclude isolates with intermediate susceptibility to the chosen antibiotic (20, 21); (ii) a change (e.g., 4-fold increase) in the MIC relative to the baseline MIC (22); or (iii) the specific mechanism that confers resistance (23, 24). Study findings regarding the association between antibiotic use and resistance may vary depending on the definition chosen. For example, Thiebaut et al. measured β-lactam antibiotic use and incidence of colonization with third-generation-cephalosporin-resistant *Enterobacteriaceae* (25). When the outcome measure was cephalosporin resistance, no correlation was found; when the outcome was limited to ESBL-producing *Enterobacteriaceae*, a significant correlation was noticed.

A second consideration in defining the outcome is whether the organism is resistant to a single drug or to more than one drug (26–28). Bacteria may exhibit coresistance to different families of antibiotics, e.g., owing to the presence of multiple resistance genes on a single transferable genetic element (29, 30). D'Agata et al. showed the differences in antibiotic exposure for subjects infected with *Pseudomonas aeruginosa* isolates resistant only to ciprofloxacin versus subjects infected with *P. aeruginosa* isolates resistant to ciprofloxacin and at least one other drug (31). For the first group, prior fluoroquinolone use was the only significant antibiotic risk factor; the second group had significant exposure to carbapenems, cephalosporins, and gentamicin in addition to quinolones. Had those authors taken only ciprofloxacin resistance into consideration (without assessing resistance to other drugs), it would falsely appear that exposure to gentamicin, for example, is a risk factor for quinolone resistance.

A third consideration when measuring resistance is choosing which types of specimens to include. The four options are (i) surveillance cultures that detect colonization (usually performed for research or infection control purposes), (ii) any clinical cultures taken during routine care of the patient (which, if positive, do not necessarily indicate infection), (iii) microbiologically and clinically documented infections (i.e., a positive culture plus signs and symptoms of infection), or (iv) site-specific cultures (e.g., blood cultures) (23, 32–34). The last three options are more commonly available, but the risk factors identified by using these samples may in fact be risk factors for developing infections rather than risk factors for harboring resistant bacteria. Only the first option, surveillance cultures, will identify asymptomatic carriers. Using the other 3 options, asymptomatic carriers will be misclassified as "controls," and the strength of the association between antibiotic use and resistance may be biased toward the null hypothesis. A key parameter is the prevalence of resistance. If resistance is rare, the probability of misclassification is low, and clinical samples may practically be used for studying antibiotic resistance. However, if resistance is common, it is preferable to use the outcome of colonization, which generally precedes infection and affects more patients (35, 36).

Finally, antibiotic resistance can be measured in several ways (37). The most common way is to measure the proportion of resistant isolates among all retrieved isolates. For example, a hospital's "antibiogram" may note that 20% of all enterococci detected in its laboratory are resistant to vancomycin. This approach is useful for the clinician who needs to prescribe empirical antibiotic therapy before the results of susceptibility testing are available. The problem with this method is that an increase in the proportion of organisms that are resistant may not necessarily reflect an increase in the absolute number (burden) of resistant organisms. An elegant demonstration of this discordance between metrics was presented by Burton et al. regarding methicillin-resistant *S. aureus* (MRSA) central line-associated bloodstream infections (BSIs) in U.S. intensive care units (ICUs) (38). The overall proportion of *S. aureus* central line-associated BSIs due to MRSA increased by 25.8% between 1997 and 2007; however, in that same time period, the incidence of MRSA central line-associated BSIs declined by half (Fig. 1). The introduction of an antibiotic will decrease the number of susceptible organisms, so the proportion of resistant organisms will increase, even if there is no increase in the number of resistant isolates. For a public health professional who is interested in the consequences of antibiotic use, knowing the burden of resistance is most important. The best way to measure the burden of resistance is by using a rate. Different ways of expressing rates are listed in Table 2.

ASSOCIATION BETWEEN EXPOSURE AND OUTCOME: DOES ANTIBIOTIC USE CAUSE ANTIBIOTIC RESISTANCE?

Although there is no doubt regarding the causal relationship between antibiotic use and resistance, defining and quantifying this

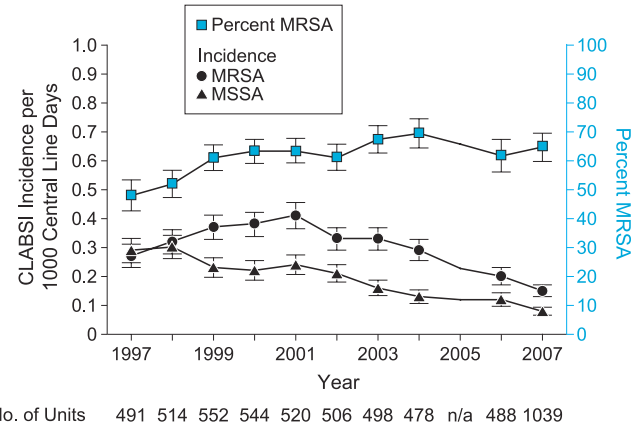

FIG 1 Trends in percent MRSA and incidence of *Staphylococcus aureus* central line-associated bloodstream infections (CLABSI) in intensive care units, from the National Nosocomial Infections Surveillance System, 1997 to 2004, and National Healthcare Safety Network, 2006 to 2007. MSSA, methicillin-susceptible *S. aureus*. (Reprinted from reference 38 with permission of the publisher. Copyright © 2009 American Medical Association. All rights reserved.)

for a given antibiotic and a given resistance are extremely difficult. In particular, it is difficult to control for all the confounding factors (known and unknown) that play a role in the development and spread of resistance. For example, different prognostic factors may influence the choice of a specific antibiotic and may also have an impact on the development of resistance ("confounding by indication"). Also, patient-to-patient transmission of resistant bacteria may be prevented by infection control measures and may be unrelated to the antibiotics prescribed. In addition, temporality may be uncertain: patients may be undiagnosed carriers of resistant bacteria before antibiotic exposure, or, on a population level, an increase in antibiotic use may be a response to an increase in antibiotic resistance rather than a trigger.

Nevertheless, research on antibiotic use and resistance supports a causal relationship by fulfilling the following criteria: (i) consistent association in different study populations, (ii) dose-effect relationships, (iii) concomitant variations (changes in antimicrobial use lead to parallel changes in the incidence of resistance), and (iv) biological plausibility based on experimental models (39).

TYPES OF RESISTANCE STUDIES

Case-Control Studies

Case-control studies begin by identifying people with a disease (cases) and people without that disease (controls) and then work backwards to determine the proportion of each group that was exposed. In case-control studies of antibiotic resistance, the disease of interest is infection (or colonization) with an antibiotic-resistant organism, and the main exposure of interest is antibiotic use. The case-control design is well suited to studying rare outcomes, such as infection with a resistant organism, better suited than, for example, a cohort study design, in which a large number of subjects would need to be recruited in order for enough of them to develop the condition under investigation (40). Likewise, a case-control study design is suitable for studying a newly encountered resistance phenotype and for outbreak investigations. Another advantage of case-control studies is that they are efficient: they take relatively little time and money to conduct because they are retrospective, and they allow for the assessment of multiple exposures.

The greatest methodological difficulty of designing a case-control study is selecting a control group. Two types of control groups are generally used in studies of antibiotic resistance: controls infected with an antibiotic-susceptible strain of the organism that appears in cases in an antibiotic-resistant form or controls uninfected with the resistant organism of interest. Studies of the first type are the most common (28, 41–95), but whether this choice is appropriate depends on the research question being posed. If the question is, "among patients with pathogen X, what are the risk factors for infection (or colonization) with a resistant strain of that pathogen?," then patients with an antibiotic-susceptible strain of the organism are suitable controls (96). For example, Lautenbach et al. (83) aimed to identify risk factors for imipenem resistance among patients with *P. aeruginosa* infection. They compared cases with imipenem-resistant *P. aeruginosa* infection to controls with imipenem-susceptible *P. aeruginosa* infection. In a multivariate logistic regression model that controlled for length of hospitalization, fluoroquinolone use in the previous 30 days was the sole predictor of imipenem resistance (odds ratio [OR], 2.52; 95% confidence interval [CI], 1.61 to 3.92).

When the research question being asked is, "what are the risk factors for acquiring antibiotic-resistant pathogen X among hospitalized patients?," the question that most studies intend to ad-

TABLE 2 Comparison of measures for reporting resistance[a]

Measure of resistance	Advantage(s)	Disadvantage(s)
Proportions	Easy to collect; useful to the clinician	Proportion-based analyses yield biased estimates from the public health perspective
Rates		
No. per unit time (for example, no. per yr)	Easy to collect and interpret	Does not account for differences in population size and therefore is not a true rate
No. per hospital bed per unit time (for example, no. per bed per yr)	Easy to collect and interpret; provides measure of density	Does not account for occupancy or turnover
No. per occupied bed (or hospital days) per unit time (for example, no. per occupied bed per yr)	Accounts for occupancy	Difficult to collect; does not account for turnover
No. per hospital admission per unit time (for example, no. per admission per yr)	Easy to collect and interpret; accounts for turnover	Does not account for occupancy

[a] Adapted from reference 37 with permission of Nature Publishing Group.

dress, choosing controls infected with antibiotic-susceptible organisms may introduce bias (40, 96). An association found between antibiotic exposure and antibiotic resistance may not reflect a true excess of antibiotic use among cases but rather may reflect decreased use among controls, since the use of an antibiotic that effectively treated their susceptible organism would prevent them from entering the control group. The effect of this selection bias will be an overestimate of the association between antibiotic use and resistance. To illustrate this principle, Harris et al. (97) measured the association between imipenem use and isolation of imipenem-resistant *P. aeruginosa* using two different control groups. When cases with imipenem-resistant *P. aeruginosa* isolation were compared to controls with imipenem-susceptible *P. aeruginosa* isolation, cases had a 27.1-times-higher odds of exposure to imipenem. When those same cases were compared to controls randomly selected from the same hospital units from which cases were drawn, the odds ratio for imipenem exposure was much lower, 6.3.

The second type of control group, patients uninfected with the resistant organism of interest, avoids the problem of overestimating the association between antibiotic exposure and resistance. A study by Fernandez et al. (98) is an example of the minority of case-control studies with this design (20, 98–109). This study compared cases with ESBL-producing *Enterobacter cloacae* isolated in clinical cultures to controls who did not have ESBL-producing *E. cloacae* isolated. In a multivariate analysis, cases had significantly higher odds of prior β-lactam use, chronic renal failure, tracheostomy, and prior hospitalization than controls.

This design has its own set of limitations. First, it cannot elucidate whether a risk factor is associated with the organism in general (*E. cloacae*) or with the resistant phenotype of the organism (ESBL-producing *E. cloacae*) in particular (40). Second, there is a risk of misclassification bias if controls who did not have clinical cultures performed were actually undetected cases; such misclassification would shift the odds ratio toward the null hypothesis (110). A proposed solution to the latter problem is to require that controls have at least one clinical culture performed. However, this requirement would introduce selection bias, in that it would eliminate patients with less severe illness, who are least likely to have cultures taken, to receive antibiotics, and to have comorbidities. To test the effect of this "severity-of-illness bias," Harris et al. (110) compared cases with imipenem-resistant *P. aeruginosa* infection to randomly selected controls and then compared the same cases to controls who had at least one clinical culture performed during their hospitalization. Odds ratios comparing antibiotic exposure between cases and controls were lower when the second control group was used. Those authors concluded that severity-of-illness bias leads to even greater underestimation than misclassification bias and advised against making clinical samples an inclusion criterion for control subjects.

To overcome the limitations inherent in the two different types of control groups, two variations on the case-control design have been developed: case-case-control (23, 33, 111–120) and case-control-control (121–126). As described by Kaye et al. (40), a case-case-control study involves two separate analyses: (i) a comparison of cases with a resistant strain of an organism to controls uninfected by that organism and (ii) a comparison of cases with a susceptible strain of the organism to those same controls. The first comparison identifies risk factors that may be relevant to both resistant and susceptible phenotypes of the organism. The second comparison yields risk factors specific to the susceptible phenotype. Comparing and contrasting the two models (qualitatively) will elucidate the risk factors specific to the resistant phenotype. Furtado et al. conducted a case-case-control study of risk factors for hospital-acquired pneumonia caused by imipenem-resistant *P. aeruginosa* (120). The first analysis (cases with imipenem-resistant *P. aeruginosa* infection versus uninfected controls) identified 7 significant risk factors for being a case: length of hospitalization; Acute Physiology and Chronic Health Evaluation II (APACHE II) score; male sex; hemodialysis; and use of corticosteroids, piperacillin-tazobactam, or third-generation cephalosporins. Of these risk factors, only corticosteroid use was also significant in the second analysis (cases with imipenem-susceptible *P. aeruginosa* infection versus uninfected controls). Therefore, those authors concluded that the other 6 risk factors were specific to the imipenem-resistant phenotype of *P. aeruginosa*.

The second variation, the case-control-control study, also includes two analyses: (i) a comparison of cases infected with a resistant strain of the organism of interest to controls uninfected by that organism and (ii) a comparison of those same cases to controls infected with a susceptible strain of the organism. The first comparison identifies risk factors that may apply to both resistant and susceptible strains of the organism. The second comparison may overestimate associations, but it isolates risk factors specific to the resistant strain. Risk factors that are statistically significant in both comparisons can be considered true risk factors for the resistant strain. Rodríguez-Baño et al. performed a case-control-control study of risk factors for bloodstream infections caused by ESBL-producing *E. coli* (126). The first analysis (cases with ESBL-producing *E. coli* versus uninfected controls) identified two risk factors for being a case: diabetes mellitus and prior use of oxyimino β-lactams (e.g., ceftazidime and ceftriaxone). Prior use of oxyimino β-lactams was also significant in the second analysis (the same cases versus controls with non-ESBL-producing *E. coli* infection), leading those authors to conclude that it is a true risk factor for ESBL-producing *E. coli* bloodstream infection.

In addition to the challenges posed by control group selection, another element to consider when designing or evaluating case-control studies is confounding. Three confounders are particularly important: time at risk, comorbid illness, and severity of illness (35, 96). For hospital-based studies, time at risk for cases or for controls with susceptible organisms is the time from admission to positive culture; time at risk for uninfected controls is the time from admission to discharge. Time at risk must be taken into consideration because patients who are hospitalized longer have more opportunities both to receive antibiotics and to acquire a resistant organism and are generally sicker. Time at risk can be controlled for by matching (time at risk for controls should be at least that for their matched cases) or statistically by multivariate analysis. The confounding effects of time at risk are evident in a study by Troillet et al. of risk factors for imipenem-resistant *P. aeruginosa* isolation (55): adding time at risk to the logistic regression model strengthened the association between imipenem exposure and positivity for imipenem-resistant *P. aeruginosa* from an odds ratio of 15.4 to 23.2. Comorbid illness and severity of present illness are 2 additional critical confounders: patients with underlying disease and/or those who are more severely ill are more likely to be exposed to antibiotics and to acquire a resistant organism. Tools such as the Charlson comorbidity index (for underlying disease) (127) and the APACHE II score (128) or the McCabe-

Jackson scale (129) (for severity of illness) can be used to measure and adjust for these two confounders in multivariate analysis. However, such tools were not designed or validated for studies of antibiotic resistance, and their suitability to this field may be suboptimal (96, 130).

As noted above, one method to control for confounding in case-control studies is individual matching. Each case is matched to one or more controls by selected confounders. For example, Soriano et al. compared risk factors for shock and mortality among cases with MRSA bacteremia versus controls with methicillin-susceptible *S. aureus* bacteremia (131). Each case was matched with one control according to underlying disease type, prognosis (based on a modified McCabe-Jackson score predicting nonfatal, ultimately fatal, or rapidly fatal disease), and length of hospitalization. Advantages of matching are as follows: (i) it is useful in studies with a small sample size, when inclusion of a large number of potential confounders in multivariate analysis will result in some strata with very few observations (132), and (ii) matching multiple controls per case increases sample size and statistical power. Disadvantages of matching are as follows: (i) it can be difficult to find a matched control for each case, particularly if matching is done for more than one variable (for this reason, matching is impracticable in case-case-control studies, which would require finding one control who matched both an antibiotic-resistant case and an antibiotic-susceptible case), and (ii) the effects of a matched variable cannot be investigated. For example, in the study by Soriano et al. mentioned above (131), being female emerged as a significant predictor of MRSA bacteremia. Had the authors matched for sex, this risk factor would not have been apparent, because matching would have artificially made the sex distribution the same for cases and controls. Matched case-control studies require statistical methods that account for matching (e.g., McNemar's test, paired *t* test, and conditional logistic regression); otherwise, the ability of matching to control for confounding is lost. Mistakes in this area are common: in a review by Cerceo et al. that identified 23 matched case-control studies of antibiotic resistance, half failed to use statistical tests that accounted for matching, yielding inaccurate results (132).

Cohort Studies

Cohort studies begin by identifying exposed individuals and unexposed individuals and then monitor both groups to determine the incidence of disease. Cohort studies of antibiotic resistance identify people exposed to an antibiotic and those unexposed and then measure the incidence of antibiotic resistance in each group. Noninterventional cohort studies are observational, meaning that researchers do not assign participants to the exposed or unexposed group (as in clinical trials); rather, participants are exposed to antibiotics or not because of decisions made as part of their clinical care.

There are two types of cohort studies: prospective and historical. In a prospective study, researchers identify exposed and unexposed groups and move forward in time to observe who develops the outcome of interest. For example, Chung et al. enrolled 119 children who had an outpatient visit for otitis media or respiratory infection; 71 had been prescribed a β-lactam antibiotic, and 48 had not been prescribed any antibiotic (133). At that initial visit and then 2 and 12 weeks later, participants had throat cultures taken to detect a marker for β-lactam resistance. At the 2-week follow-up, 67% of children who had been given antibiotics had the resistance marker, compared to 36% of children who had not been given antibiotics (risk ratio [RR] = 1.9; CI = 1.3 to 2.7); at 12 weeks, there was no longer a difference in resistance between the exposed and unexposed groups.

Instead of comparing exposed and unexposed groups, some cohort studies compare groups with different types or amounts of exposure. A prospective cohort study by Harbarth et al. compared patients who received prophylactic antibiotics after coronary artery bypass graft (CABG) surgery for less than 48 h versus more than 48 h and monitored them to measure two outcomes: incidence of surgical site infection and isolation of cephalosporin-resistant *Enterobacteriaceae* or VRE (134). In logistic regression models that controlled for confounders such as length of hospital stay, compared to patients who received prophylaxis for shorter durations, patients who received prophylaxis for longer durations had the same risk of surgical site infection but a higher risk of antibiotic resistance.

For outcomes that may take years to develop, prospective cohort studies will take years to complete. A faster alternative is the historical cohort study: researchers identify the exposed and unexposed groups from historical data (such as past medical records) and assess the outcome when the study is begun (135–139). Schwaber et al. used a historical cohort design to compare the risk of exposure to two different antibiotics on acquisition of cephalosporin-resistant *Enterobacter* sp. isolation (135). Those researchers reviewed hospital databases to identify all patients who had been treated with either piperacillin-tazobactam (exposure type 1) or a broad-spectrum cephalosporin (exposure type 2) during the 38 months defined as the study period. Next, those researchers reviewed subjects' medical records beginning at the start of antibiotic therapy to determine who had a subsequent clinical culture positive for cephalosporin-resistant *Enterobacter* spp. A multivariate logistic regression model demonstrated no difference in the risk of *Enterobacter* sp. acquisition in the two exposure groups.

Cohort studies have several advantages. First, they allow for the assessment of more than one outcome, as in the CABG study that measured the incidence of both surgical site infection and antibiotic resistance (134). They are also valuable for studying a spectrum of resistant organisms, as in a historical cohort study by Carmeli et al. that analyzed resistance patterns of 10 nosocomial pathogens after treatment with ceftriaxone versus ampicillin-sulbactam (139). Second, in a prospective cohort study, the temporal sequence is easy to establish; that is, the exposure definitely preceded the outcome.

Cohort studies are also subject to limitations. Because subjects are not assigned to an exposure group at random, there is potential for confounding. For example, in the study by Schwaber et al. (135), if doctors tended to prescribe piperacillin-tazobactam to patients who were more severely ill, an association found between that drug and acquiring a resistant organism could reflect these patients' poorer health and not the type of antibiotic that they received ("confounding by indication"). In their regression model, those authors controlled for variables that they found to be significantly different between the 2 groups (including sex, certain comorbidities, having a surgical procedure, and being in the ICU). Nevertheless, there is always a risk of residual confounding by unmeasured or imperfectly measured variables. A second problem with cohort studies is that a large number of subjects may need to be enrolled so that enough of them develop the outcome

being investigated. Acquisition of a resistant organism is an uncommon event (resistant *Enterobacter* appeared in 2% of patients in the study by Schwaber et al., for example), which may explain why the cohort design is infrequently used to study the association between antibiotic use and resistance. Prospective cohort studies are often noted for their long duration and expense.

Ecological Studies

Ecological studies examine the association between antimicrobial exposure and resistance at the group level, using a correlation coefficient based on aggregate data as the measure of association (140–145). Different types of groups can be used as the unit of analysis. For example, in a study of 20 industrialized countries, Albrich et al. found a positive correlation at the national level between total outpatient antibiotic consumption and penicillin nonsusceptibility in *S. pneumoniae* and between macrolide consumption and macrolide resistance in *S. pneumoniae* and *S. pyogenes* (142). At the regional level, Bergman et al. examined 18 districts in Finland and found an association between the amount of macrolide use (based on sales from wholesalers to pharmacies) and the proportion of *S. pyogenes* isolates resistant to erythromycin in the following year (145). At the hospital level, Fridkin et al. demonstrated an association between vancomycin and third-generation cephalosporin use and the prevalence of VRE in adult ICUs (140). At the community level, MacDougall et al. found an association between outpatient fluoroquinolone use and resistance in *E. coli* in nearby hospitals (144).

Ecological studies have several advantages. First, by relying on existing data sets that can be relatively easily obtained and linked at the aggregate level, these analyses are usually inexpensive, easy to perform, and reproducible. Second, because they cover broad geographical areas or time frames, they reflect large variations in the types and amounts of antibiotics used; therefore, results can be easily generalized. Third, in contrast to approaches based on individual-patient-level data, these studies may be able to express the total effect of antibiotic exposure, including transmission of resistant bacteria. This is important, because the total effects of antibiotics encompass not only the direct effects on the individual who receives the antibiotic but also the indirect effects mediated by effects on transmissibility or on the likelihood of transmission of susceptible organisms (3, 146).

Critics of ecological studies point to the major limitation of group-level analyses, which is the failure to allow causal inferences at the individual level. To wit, if we see an association between antibiotic consumption and resistance at the group level, it would be an "ecological fallacy" to assume that the individuals exposed to antibiotics were the ones with resistant bacteria (147). The converse is also true. Harbarth et al. performed a parallel analysis of individual and aggregated data concerning antibiotic exposure and resistance in Gram-negative bacilli (3). At the hospital level, there was no association between the use of fluoroquinolones, third-generation cephalosporins, ampicillin-sulbactam, or imipenem and the proportion of isolates resistant to these antibiotics, while at the individual level, there was a strong association. Similarly, Donnan et al. found a strong association between individual-level exposure to trimethoprim or to other antibiotics and the presence of trimethoprim-resistant bacteria in individual patients' urine samples that was obscured by analysis of aggregate-level data from the same population (148). In other words, presuming individual-level relations on the basis of group-level data can lead to distorted conclusions and should be avoided.

Another limitation of applying group-level analysis to longitudinal antibiotic use and resistance data is that a temporal relationship between exposure and outcome cannot be assumed, meaning that an increase in antimicrobial consumption over time might actually be the consequence of, and not the reason for, the increased incidence of resistant bacteria. This potential pitfall in ecological studies has been coined "temporal ambiguity" (146). This pitfall can be addressed in part by modeling antimicrobial use measurements and antimicrobial resistance measurements as time series (see the section on modeling below).

Nonrandomized Intervention (Quasiexperimental or Before-After) Studies

Quasiexperimental studies are used to evaluate interventions when randomization is not feasible. In research on antimicrobial resistance, quasiexperimental studies measure the effect of antimicrobial stewardship interventions (e.g., restricting antibiotics or formulary changes) on antimicrobial resistance. Quasiexperimental studies can measure exposure and outcomes at either the individual or population level. However, in the field of antimicrobial resistance, these studies are typically at the population level. In this way, they resemble ecological studies, the main difference being that ecological studies are mostly of an observational nature, and quasiexperimental studies include an intervention (intended or not intended).

There are several types of quasiexperimental study designs (Fig. 2). They can be grouped by whether or not they include a control group and/or a pretest phase (i.e., one or more measurements before the intervention). What follows are examples of quasiexperimental designs that are commonly used to study antibiotic resistance (149).

In the pretest-posttest design, one or more measurements are taken before and after the intervention; there is no control group that has not undergone the intervention for comparison (150–153). For example, Di Pentima and Chan used this design to study the effect of limiting vancomycin use on the incidence of VRE in a pediatric hospital (152). They measured vancomycin doses per 1,000 patient-days and the incidence of VRE in the year before an antibiotic stewardship program (ASP) was implemented. In the 2 years after the start of the ASP, which required that physicians list an approved indication for vancomycin, both vancomycin use and the incidence of VRE decreased. That study illustrates a major limitation of quasiexperimental research: the difficulty in controlling for confounders. Unmeasured variables such as improved hand hygiene or attention to contact precautions, not only the ASP, may have contributed to the decline in VRE incidence observed in that study. This limitation can become especially problematic in interpreting research on antibiotic resistance, because restrictions on antibiotic use are often implemented as part of a bundle that includes additional infection control measures. Also, it is important to consider the timing of the intervention; if the intervention was initiated in response to a marked increase in resistance, one may expect a decline in resistance simply by regression to the mean and not because of the intervention.

A quasiexperimental design that attempts to control for confounding is the one-group pretest-posttest design with a nonequivalent dependent variable (154, 155). For example, Gottesman et al. observed a decline in fluoroquinolone-resistant *E. coli* in

Quasi-experimental designs that do not use control groups

1. The one-group pretest-posttest design:
   O1 X O2
2. The one-group pretest-posttest design that uses a double pretest:
   O1 O2 X O3
3. The one-group pretest-posttest design that uses a nonequivalent dependent variable:
   (O1a, O1b) X (O2a, O2b)
4. The removed treatment design:
   O1 X O2 O3 removeX O4
5. The repeated treatment design:
   O1 X O2 removeX O3 X O4

Quasi-experimental designs that use control groups and pretests

1. Untreated control group design that uses dependent pretest and posttest samples:
   O1a X O2a
   O1b O2b
2. Untreated control group design that uses dependent pretest and posttest samples and a double pretest:
   O1a O2a X O3a
   O1b O2b O3b
3. Untreated control group design that uses dependent pretest and posttest samples and switching replications:
   O1a X O2a
   O1b O2b X O3b

* O = Observational measurement, X = Intervention under study, and time moves from left to right

FIG 2 Types of quasiexperimental study designs used in antibiotic resistance research (Modified from reference 149 with permission of the Infectious Diseases Society of America.)

urine cultures during a community intervention in which preapproval was required to prescribe ciprofloxacin (155). They also measured a nonequivalent dependent variable: *E. coli* resistance to two other antibiotics whose use was not restricted. If resistance to both fluoroquinolones and the nonrestricted drugs had declined, we might suspect that something other than the intervention (which was specific to ciprofloxacin) was responsible for the decline. The observed absence of change in the nonequivalent dependent variable supported a causal relationship between ciprofloxacin restriction and increased susceptibility to fluoroquinolones.

Quasiexperimental designs in which the intervention is removed (with or without repeat intervention) also address the problem of confounding (155, 156). When an outcome disappears after the intervention is removed, we are more confident of a causal relationship between the two. For example, 7 months into the study by Gottesman et al. described above (155), the effect of restricted use of ciprofloxacin was cancelled out when generic ofloxacin was added to the formulary. As fluoroquinolone use increased to preintervention levels, the proportion of *E. coli* isolates susceptible to fluoroquinolones also returned to baseline (Fig. 3).

In the two-group pretest-posttest design, the study sample includes an intervention group and a control group. Although group allocation is not random, the pretest measurements tell us whether the two groups are similar. Therefore, if a change between the pre- and the posttest measurements is observed only for the intervention group, the assumption of causal inference is more robust than it would be had there been no control group for comparison. Charbonneau et al. used this design to investigate the association between fluoroquinolone use and MRSA among hospitalized patients (157, 158). This study consisted of 4 hospitals with similar baseline proportions of *S. aureus* isolates that were methicillin resistant. An intervention consisting of restriction of fluoroquinolone use was carried out in only one of them. At the end of the intervention period, a significant reduction in the proportion of MRSA occurred in the intervention hospital and not in the control hospitals.

Although adding a pretest or a control group can help to overcome the problem of confounding in quasiexperimental studies, there is always a risk of unidentified confounders in studies that lack randomization. In particular, the intentional decreased use of a particular class of antibiotics often leads to an increased use of another class; accordingly, the effect on resistance might be due to the decreased use of class A or the increased use of class B. This phenomenon occurred in a before-after study by Rahal et al., in which one hospital's efforts to restrict cephalosporin use led to both a reduction in cephalosporin use and an increase in imipenem use (159). It cannot be determined whether the observed drop in the incidence of nosocomial ESBL-producing *Klebsiella* was triggered by the increase in imipenem use or the restriction of cephalosporins. Its limitations notwithstanding, the quasiexperimental study design is useful in situations in which randomization is not possible because of (i) ethical considerations, (ii) the inability to randomize individual patients or locations, or (iii) a need to intervene quickly (hence, the intervention is undertaken in the context of clinical care and only retrospectively evaluated as research) (149).

Randomized Controlled Trials

Randomized controlled trials (RCTs) are considered the gold standard for estimating the true effect of an intervention. The main feature of these studies is that individuals (or groups of individuals) are randomly assigned to either an intervention or a control group. This process of random assignment minimizes un-

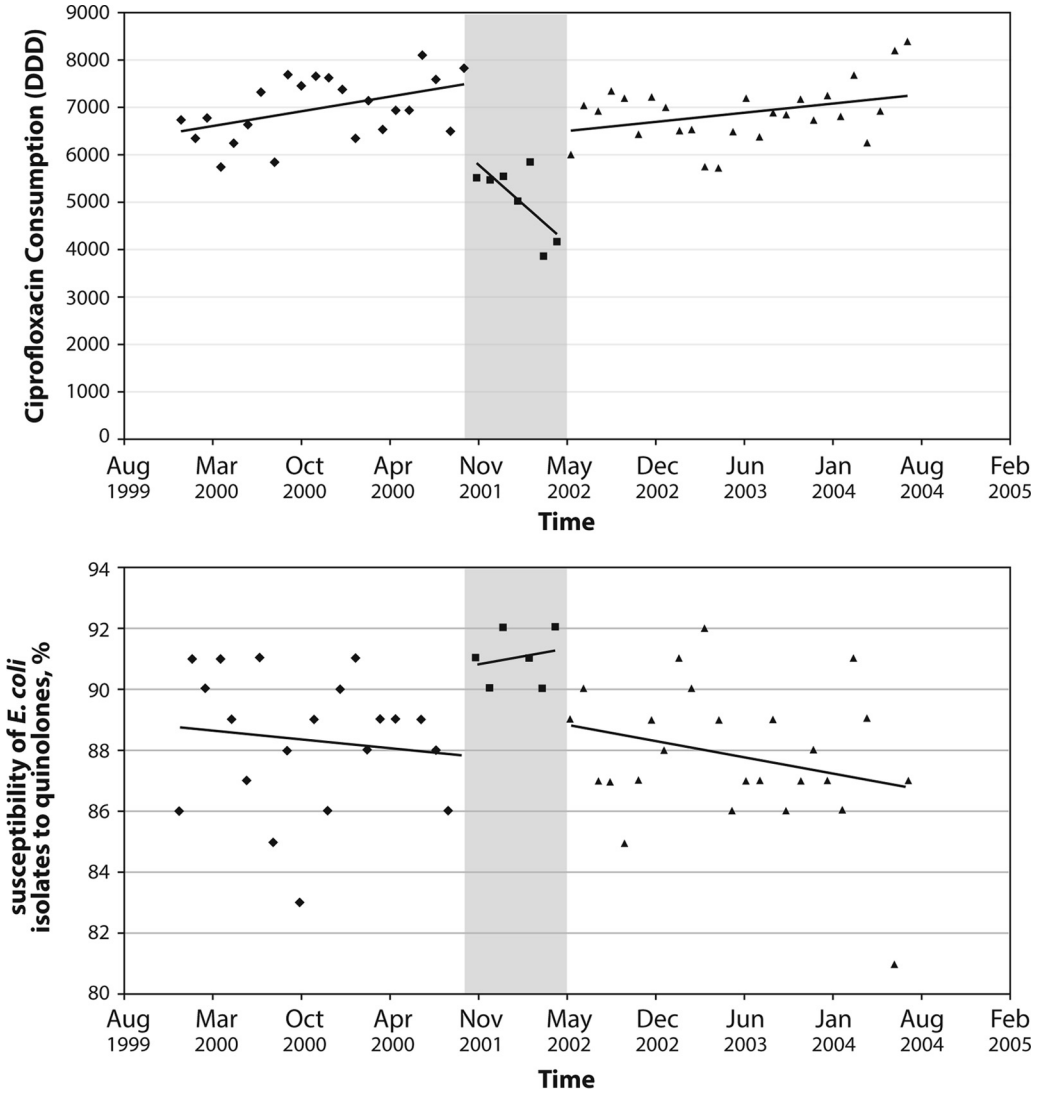

FIG 3 Relationship between quinolone consumption (top) and susceptibility of *E. coli* isolates from urine cultures to quinolones (bottom), by month. The shaded area is the intervention period; to the left is the preintervention period, and to the right is the postintervention period. DDD, defined daily dose. (Modified from reference 155 with permission of the Infectious Diseases Society of America.)

noticed systematic differences between the groups except for the intervention being studied and ensures that the resulting difference in outcomes between the groups is due to the intervention itself and not to other factors. RCTs may provide the strongest evidence for a causal connection between antimicrobial consumption and resistance (160).

Randomization can be performed at the individual level or at the group level. At the individual level (parallel group randomization), each participant is randomly selected for a group, and all the participants in the same group receive (or do not receive) an intervention. For example, Malhotra-Kumar et al. randomized 224 healthy volunteers to receive a full course of azithromycin, clarithromycin, or placebo. The primary outcome was changes in the proportions of macrolide-resistant streptococci in pharyngeal swabs (160). Beerepoot et al. randomly assigned 221 premenopausal women with recurrent urinary tract infection to 12-month prophylactic use of either trimethoprim-sulfamethoxazole (TMP-SMX) or cranberry capsules (161). The primary outcome measure was the percentage of endogenous *E. coli* isolates resistant to TMP-SMX in each of the study groups at baseline, during prophylaxis, and 3 months after discontinuation of the study medication.

At the group level (cluster randomization), each group of participants is randomly assigned to receive (or not receive) an intervention. For instance, Skalet et al. randomly assigned 24 communities in Ethiopia to receive either immediate (at months 0, 3, 6, and 9) or delayed (after month 12) mass azithromycin treatment of children for trachoma control (162). They compared the prevalence of macrolide resistance in nasopharyngeal *S. pneumoniae* isolates in the immediate-treatment group (pre- and posttreatment) with that in the delayed-treatment group (pretreatment).

Cluster randomization is particularly useful when the intervention involves exposure to antibiotics because of the potential for ecological effects; i.e., via transmission, antibiotics may affect resistance patterns even in control group patients who did not receive the antibiotic (163). Accordingly, many RCTs in the field of antibiotic resistance use cluster randomization. Units of random-

ization may vary, including, for example, patient care units (164, 165) and communities (162). One drawback of performing cluster randomization is the reduced statistical efficiency, which mandates recruitment of more participants in order to obtain the same statistical power as individual randomization. Another major limitation of cluster randomization is the similarity that may exist between subjects in the same group (e.g., patients in the same ward), which may have an impact on the outcome. To overcome this problem, de Smet et al., in an RCT evaluating the effect of digestive tract decontamination regimens on infection incidence in 13 Dutch hospitals, used a crossover study design, wherein each unit of randomization was assigned to all interventions in rotation (164). In that study, the three interventions were selective digestive tract decontamination (SDD), selective oropharyngeal decontamination (SOD), and standard care; each ICU carried out each intervention for 6 months. This design controlled for unit-specific characteristics, for example, differences in hand hygiene or isolation practices between units. Another way to correct for the nonindependence between individuals in the same unit is by using the appropriate statistical analytical approach (see the section on multilevel analysis below).

Proper randomization in RCTs is critical in order to prevent allocation bias; i.e., participants should not be assigned intentionally to a specific treatment group. Proper randomization permits equal distribution of all potential confounders (known and unknown) between the study groups, so their effects on the results are negligible. For example, de Jonge et al. assessed the effect of SDD on the acquisition of resistant bacteria among adult patients admitted to 2 ICUs; one unit was designated the SDD unit, and one was designated the control unit (165). The baseline characteristics were similar in the two groups, including the number of patients who were colonized with resistant bacteria at inclusion.

RCTs have the advantage of providing clear temporal sequences between antibiotic exposure and development of resistance. In many studies, the prevalence of resistant organisms is determined upon study admission and at repeated intervals during the intervention. A change in prevalence of resistant organisms during the study period suggests a temporal relationship between the use of antibiotics and development of resistance.

Despite being the "gold standard," RCTs have some disadvantages. Properly conducted RCTs are demanding in terms of time, labor, and money. RCTs are generally brief, and there may not be enough time for changing trends in antibiotic resistance that result from the intervention to become apparent. Finally, the results of an RCT may not be generalizable to settings with markedly different baseline prevalences of resistant organisms. Silvestri and van Saene noted that in settings where MRSA is endemic, there was a trend toward increased MRSA infections in patients receiving SDD, whereas SDD had no impact on MRSA infections in settings with low MRSA prevalence (166).

Compared to observational studies, there are relatively few RCTs that measure the association between antibiotic use and resistance. This is a missed opportunity, because phase 3 trials of new antibiotics, with their meticulous follow-up, are an excellent (but underused) context for studying this topic. An RCT comparing ertapenem to piperacillin-tazobactam for complicated intra-abdominal infections included acquisition of resistant bowel flora as a secondary outcome (167). Rectal swabs were taken at the start and end of treatment. No ertapenem-resistant *Enterobacteriaceae* were identified among patients in the ertapenem arm, while eight patients on piperacillin-tazobactam acquired *Enterobacteriaceae* resistant to that drug. Just as all drug trials are required to assess and report adverse events, we recommend that assessment of resistance be included in phase 3 trials.

Systematic Reviews and Meta-Analyses

Systematic reviews collate and synthesize data from all studies on a given research question that fulfill predefined eligibility criteria. The "predefined" aspect is critical: formulating explicit methods for choosing which studies to include in a systematic review prevents the bias that may enter traditional narrative reviews, in which authors may selectively present evidence that supports their viewpoint (168). Meta-analysis is a type of systematic review that uses statistical techniques to pool the results of many studies and arrive at a single quantitative summary (a weighted average). Whether or not researchers perform a meta-analysis in addition to a systematic review depends on the heterogeneity of the studies; it may not be appropriate to pool results from studies whose designs, participants, exposures, or outcome measures differ markedly.

Systematic reviews and meta-analyses are particularly helpful for sorting through conflicting evidence. A meta-analysis by Bliziotis et al. examined whether aminoglycoside and beta-lactam combination therapy is less likely to cause antibiotic resistance than beta-lactam monotherapy (169). Dual therapy with aminoglycosides and beta-lactams gained favor after animal studies in the 1980s suggested a preventive effect on resistance, but later clinical studies failed to confirm a benefit. Those authors identified 8 RCTs comparing dual therapy to beta-lactam monotherapy. Less resistance appeared in the dual-therapy group in 2 trials and in the monotherapy group in 6 trials, although the difference was not statistically significant in any of them. In the meta-analysis, patients receiving monotherapy had a 10%-lower risk of developing resistance, but the difference was not statistically significant (95% CI, 0.56 to 1.47), indicating that neither form of therapy is more protective against resistance than the other.

A benefit of meta-analyses is that, by combining samples from several studies, there is greater statistical power. Thus, differences that were undetectable in individual small studies may become evident in meta-analyses. For example, Tacconelli et al. conducted a meta-analysis of the association between cephalosporin use and subsequent isolation of MRSA (170). Five of the six studies included in the meta-analysis failed to demonstrate an association, but the meta-analysis found a statistically significant association between cephalosporin use and MRSA isolation (RR, 2.21; 95% CI, 1.70 to 2.88).

Systematic reviews and meta-analyses have several potential pitfalls. First, their quality is only as good as the quality of the individual studies that they include. Therefore, defining minimum requirements for quality is a crucial aspect of study selection. Costelloe et al. performed a meta-analysis of studies of antibiotic resistance among individuals prescribed antibiotics in primary care (171). They specified that studies must meet at least 3 out of 5 quality criteria: reliable measures of antibiotic exposure and resistance, unbiased control selection, ability to identify incident cases (i.e., patients were known to be culture negative for resistant bacteria before receiving antibiotics), and adjustment for key confounders. Of 24 relevant studies, Costelloe et al. excluded 3 for poor quality; such culling strengthens the results of their meta-analysis.

A second potential pitfall is publication bias. Authors are more likely to submit and editors are more likely to publish studies with positive findings (172). Hence, meta-analyses that include only published studies may arrive at summary measures that overestimate the true effect size. One step to overcome publication bias is to search databases of gray (unpublished) literature such as the Thomson Reuters Web of Knowledge (which includes conference abstracts) and clinical trial registries. Once a meta-analysis is completed, statistical techniques can be used to test for publication bias. The most common is the funnel plot, in which study size (on the *y* axis) is plotted against effect size (on the *x* axis). When no publication bias is present, the graph will resemble a funnel: large studies will cluster in the middle (near the true effect size), and a similar number of smaller studies will be present on either side (randomly over- or underestimating the true effect size). When publication bias is present, there will be a hole in the lower left corner, signifying the absence of studies with a small sample size and no or negative effects (168). Other statistical techniques to test for publication bias include contour-enhanced funnel plots, Egger's test, and the trim-and-fill method (173).

A third pitfall occurs when heterogeneous studies, which should be reported in the format of a systematic analysis, are erroneously pooled in a meta-analysis. Clinical heterogeneity may be obvious. For example, Sibanda et al. identified 8 good-quality studies that examined the association between the use of prophylactic trimethoprim-sulfamethoxazole among HIV-positive individuals and the development of resistance to other classes of antibiotics (174). Those researchers correctly avoided a meta-analysis because of the noncomparability of the study populations (inpatients and outpatients, infants of HIV-positive mothers, children, and adults) and the outcomes (ranging from VRE colonization to MRSA bacteremia).

Another way to handle clinical heterogeneity is to perform subgroup analyses of studies that can logically be pooled. For example, the study of antibiotic resistance among primary care patients by Costelloe et al. presented separate meta-analyses based on infection type (respiratory or urinary tract) and the interval between antibiotic exposure and assessment of resistance (171). It is also informative to perform subgroup analyses when studies are methodologically heterogeneous. Carmeli et al. identified 15 case-control studies that compared vancomycin exposure between VRE-colonized or -infected cases and VRE-negative controls (175). A major methodological difference in those studies was that 5 of them adjusted for length of hospitalization and 10 did not. The pooled odds ratio for the studies that adjusted for length of hospitalization was much lower and not statistically significant (OR, 1.4; 95% CI, 0.74 to 2.6) compared to the pooled odds ratio for studies that did not adjust for this confounder (OR, 3.1; 95% CI, 1.8 to 5.3). These subgroup analyses illustrate that failure to control for the confounding effects of length of hospitalization may result in an overestimation of the association between vancomycin use and VRE acquisition. The clinical implication of this finding is that the popular strategy of limiting vancomycin use to reduce VRE incidence is unlikely to be effective.

Even studies that are clinically and methodologically similar may be statistically heterogeneous; i.e., their results may differ by more than their variation in sampling can explain. Tests to identify statistical heterogeneity include Cochran's Q and I^2 (176). When heterogeneity is present, options to address it include performing subgroup analyses, using a random-effects model to calculate the summary estimate, or a metaregression, a statistical technique to identify factors that influence the studies' outcome (i.e., that contribute to the heterogeneity) (177). Tacconelli et al. detected significant heterogeneity in their meta-analysis of studies of the association between antibiotic use and MRSA colonization or infection (170). A metaregression revealed that the main source of difference in study results was whether researchers measured antibiotic exposure during a period of greater or less than 180 days prior to MRSA isolation. This ability to pinpoint the reasons for inconsistent results between studies is another strength of meta-analysis (168).

The Centre for Evidence-Based Medicine ranks systematic reviews and meta-analyses (of randomized controlled trials) as the highest level of evidence (178). When performed well, systematic reviews and meta-analyses are powerful because they assess and distill a large body of knowledge into a concise "bottom line" (168). Careful consumers of these studies in the field of antibiotic use and resistance should evaluate whether the authors have ensured the quality of studies included in their analyses (for example, adjustment for potential confounders such as severity of illness, comorbidity, and length of hospitalization) and that meta-analyses have not pooled studies of markedly different antibiotic exposures and resistance outcomes.

Studies Based on Mathematical Modeling

Mathematical modeling uses theoretical assumptions and mathematical tools in order to evaluate the real-world dynamics between antibiotic use and the spread of antibiotic resistance. Traditional epidemiological studies of resistant organisms examine individual patient-level associations between antibiotic use and colonization or infection with resistant organisms, using statistical concepts assuming independence between subjects (i.e., logistic regression analysis). In contrast, modeling studies can take into account transmission effects and indirect effects of antibiotic use by using a mix of individual- and group-level data. In the case of transmissible diseases, this avoids the problem of erroneously assuming independence of events and may also mitigate confounding.

Another main advantage of modeling is its ability to test hypotheses and interventions that may be experimentally impossible, unethical, or expensive or when results of intervention trials are confusing or difficult to interpret. For example, Bergstrom et al. pointed out that studies of antibiotic cycling have yielded mixed results, perhaps because of the comparison to historical controls or uncontrolled confounding by concomitant infection control interventions (179). To overcome these limitations, they developed a mathematical model of antimicrobial cycling in a hospital setting to explore the association between cycling and resistance. According to their model, cycling was less effective than mixing antibiotics in reducing the evolution and spread of antibiotic resistance. Another example of using modeling to guide clinical practice is a study by D'Agata et al. that aimed to define the optimal antibiotic treatment strategy to prevent the emergence of resistance (180). They found that early initiation of treatment and combination therapy with two antibiotics prevented resistance most effectively.

Modeling is especially attractive for studying antibiotic resistance since it spares the time needed for the evolution of resistance. It is also suitable for preparing for emerging antibiotic-resistant pathogens, where accurate data are not available. For

1. Are the study aims clearly stated?
2. What is the study design and is it appropriate for the aims?
3. What is the exposure variable and how is it measured? (Defined Daily Dose or other measures? Exposure to any antibiotic, a specific class, or a specific drug? Categorical [yes or no] or continuous? If categorical, was a minimum length of therapy defined? What was the time frame for exposure (e.g. antibiotic use during the current hospitalization, in the past 3 months, etc.)?
4. What is the outcome variable and how is it measured? (Resistance in general or resistance by a specific mechanism Was resistance to more than 1 drug considered? Was resistance measured in clinical cultures, surveillance cultures, or both? Was resistance measured as a proportion or as a rate?)
5. What is the study sample?
   - (For cohort) Are the exposed and unexposed groups similar regarding important potentially confounding variables?
   - (For case-control) Who are the controls? Is this the best control group for the research question posed?
6. Did the researchers control for possible sources of confounding? (e.g. co-morbid illness, severity of illness and time at risk)
7. What are possible sources of bias?

FIG 4 Suggested guidelines for evaluating studies examining the effect of antibiotic usage on resistance.

example, modeling could be used to weigh the health and economic advantages of universal MRSA decolonization with mupirocin against the risk of mupirocin resistance that this strategy might confer (181).

Mathematical models have several pitfalls. First, the trade-off that exists between simplicity and accuracy of the model sometimes leads to a model that oversimplifies reality. For example, in the study of antibiotic cycling with two drugs by Bergstrom et al. discussed above, the model did not include the possibility of resistance to both drugs, which in fact is quite common among hospitalized patients (179). A second pitfall is that models are based on assumed parameter values, for example, the level of physician compliance with antimicrobial cycling programs (179). Since these parameters may be important factors in evaluating the intervention, results may be qualitatively appropriate but not quantitatively exact. Finally, models most frequently lack prospective validation.

Choosing the appropriate type of model may overcome some of the weaknesses described above. In probabilistic (stochastic) models, variables are described by probability distributions and not by fixed values (as in deterministic models). Haber et al. used a probabilistic model to analyze the effects of switching to second-line antibiotics on the incidence of nosocomial infections caused by bacteria resistant to first-line antibiotics (182). In dynamic models, the element of time is incorporated into the model. An example of a situation in which this is important is the decolonization therapy mentioned above: over time, susceptibility to the decolonizing agent may decrease, and therefore, the effectiveness of this strategy will wane. A dynamic model takes this change into account (183).

STATISTICAL METHODS TO CONTROL FOR CONFOUNDING

Multilevel Analysis

Incorporating both individual-level and group-level measures in the same analysis (multilevel analysis) allows better control for confounders, since individual-level analyses alone may incompletely capture the population-level effects of antibiotics. Moreover, it is conceptually appropriate for investigating the association between antibiotic exposure and resistance because group factors, such as "colonization pressure" or personnel compliance with infection control measures, may influence the individual risk of acquiring resistant organisms (184). This approach may also allow adjusting for clustering effects. Muller at al. used multilevel analysis specifically to explore the interactions between antibiotic pressure at both individual and group levels and MRSA isolation (185). They found that individual exposure to fluoroquinolones and collective exposure to penicillins (at the hospital ward level) increase the risk of MRSA isolation, after adjustment for potential confounders such as age, sex, MRSA colonization pressure, and type of hospital ward. Therefore, the total burden of antibiotic consumption in a population affects the individual's risk of acquiring resistant organisms beyond that conferred by that individual's antibiotic consumption.

Propensity Score

Another statistical method to control for confounding in the absence of proper randomization of study participants is the construction of a propensity score, i.e., defining the individual composite risk based on nonantibiotic covariates (15, 23, 108, 186, 187). Wener et al. used the propensity score, based on nonantibiotic variables, to express each patient's probability of being infected with *Klebsiella* spp. (23). This scoring was incorporated into a multivariate analysis including the antibiotic variables in order to analyze the association between antibiotic exposure and isolation of ESBL- and non-ESBL-producing *Klebsiella* spp. Although theoretically, a propensity score provides the advantage of control for confounding by indication, a systematic review of the application of propensity score methods failed to yield evidence of substantially different estimates using propensity scores compared with conventional multivariable methods (188).

Time Series Analysis

A practical method for analyzing pooled longitudinal data of antibiotic use and resistance is time series analysis. This technique takes into account the influence of time in the relationship, thereby guaranteeing that the suspected cause precedes the effect.

It also accounts for the possible dependence between repeated outcome measures, as may be true for the level of resistance observed over time. Lopez-Lozano et al. applied this design to analyze hospital antibiotic use and resistance data; they were able to demonstrate a temporal relationship between hospital ceftazidime use and the percentage of ceftazidime-resistant/ceftazidime-intermediate Gram-negative bacilli and between hospital imipenem use and the percentage of imipenem-resistant/imipenem-intermediate *P. aeruginosa* (189). Another study, by Aldeyab et al., demonstrated temporal associations between the use of specific antibiotics and infection control measures and the incidence of MRSA in the hospital (190).

Time series methods are particularly useful in quasiexperimental study designs in which infection rates have been ascertained before and after an intervention (190, 191). However, because the *a priori* hypotheses are less well specified than in the situation of a defined intervention, there may be a problem of multiple statistical hypothesis testing with spurious findings that are not always biologically plausible ("statistical fishing expedition"). The fitted regression coefficients are also not easily interpreted, in part because they are not translatable into familiar measures of relative risk (192).

CONCLUDING REMARKS

Studies linking antimicrobial use to antimicrobial resistance are almost as old as antibiotics themselves. Although the association between antimicrobial use and resistance is well accepted, a direct causal relationship is difficult to demonstrate using any specific study design. Careful reading of the literature connecting antimicrobial use with resistance should take heed of the following potential pitfalls in interpretation: study design and its suitability to study aims, definitions of exposure and outcome measures and metrics used, control group selection, and confounders (Fig. 4). Awareness of all of these elements is vital for adequate epidemiological interpretation of studies examining the effect of antibiotic use on resistance.

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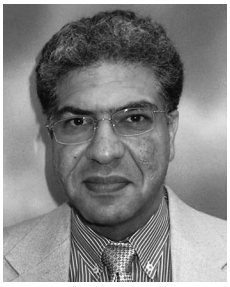
Vered Schechner, M.D., is a senior physician in the Division of Epidemiology and Preventive Medicine at the Tel Aviv Sourasky Medical Center in Tel Aviv, Israel. Dr. Schechner received her M.D. degree from Tel Aviv University. She did her residency at Beilinson Medical Center, Petach Tikva, Israel, and a fellowship in infectious diseases at the Tel Aviv Medical Center. In her position, Dr. Schechner is involved in the everyday practice of hospital epidemiology and has conducted research on predictors of *Pseudomonas* infection among patients with bacteremia upon hospital admission and on the epidemiology of KPC-producing *Klebsiella pneumoniae*. Her main research interests are in the field of antimicrobial prescribing and the epidemiology of antimicrobial resistance.

Elizabeth Temkin, M.S.N., Dr.P.H., is an epidemiologist in the National Center for Infection Control of the Israel Ministry of Health and in the Division of Epidemiology and Preventive Medicine at the Tel Aviv Sourasky Medical Center. Dr. Temkin received a master's degree from Yale School of Nursing and a doctorate from Johns Hopkins School of Public Health. She conducts research and surveillance regarding hospital-acquired infections, antibiotic use, and antibiotic resistance.

Stephan Harbarth, M.D., M.S., is an associate professor and hospital epidemiologist at Geneva University Hospitals in Geneva, Switzerland. He also serves as senior attending physician in geriatric and general infectious diseases. Dr. Harbarth earned his medical degree from Ludwig-Maximilians University in Munich, Germany, and completed his residency in internal medicine and tropical medicine at Munich University Hospitals. After serving as a clinical fellow in the Infectious Diseases Division and Infection Control Program in the Department of Internal Medicine at Geneva University Hospitals, Dr. Harbarth completed his master's degree in epidemiology at Harvard University in Cambridge, MA. He is board certified in infectious diseases. Dr. Harbarth's work has garnered several awards, including the Interscience Conference on Antimicrobial Agents and Chemotherapy Young Investigator Award from the American Society for Microbiology in 2003, the Young Investigator Award from the European Society of Clinical Microbiology and Infectious Diseases in 2006, the Swiss Society for Infectious Diseases Award for epidemiological research in 2008, and the Society of Healthcare Epidemiology of America Investigator Award in 2011. A prolific author, Dr Harbarth has written or coauthored more than 200 scientific publications and is coeditor of several peer-reviewed journals. He is a member of many international societies, working groups, advisory boards, and tasks forces, including the WHO and ECDC.



Yehuda Carmeli, M.D., M.P.H., professor of medicine, Tel Aviv University, is the Head of the Israeli National Center for Antibiotic Resistance and the chief of the Division of Epidemiology and Preventive Medicine at the Tel Aviv Sourasky Medical Center in Tel Aviv, Israel. He is also on the research staff at the Division of Infectious Diseases, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA. Dr. Carmeli received his M.D. degree from Ben Gurion University, Israel, and his M.P.H. degree at Harvard School of Public Health. He served his residency at Hadassah Medical Center, Hebrew University, Jerusalem, and then served as a fellow in Medicine at Massachusetts General Hospital, Boston, MA. Dr. Carmeli completed his fellowship in Infectious Diseases at Beth Israel Deaconess Medical Center, Boston, MA. His primary research interest is the epidemiology of resistance to antibiotics. Dr. Carmeli is the author of over 200 research articles, the recipient of multiple research grants and awards, and a member of editorial boards of major journals in the fields of infectious diseases and antibiotic research.

Mitchell J. Schwaber, M.D., M.Sc., is the director of the National Center for Infection Control of the Israel Ministry of Health. A native of Boston, MA, he pursued his medical studies at the Albert Einstein College of Medicine, medical residency at Massachusetts General Hospital and Hadassah University Hospital in Jerusalem, and training in infectious diseases and epidemiology at Beth Israel Deaconess Medical Center and the Harvard School of Public Health. In addition to his duties with the Ministry, he practices internal and infectious disease medicine at Tel Aviv Sourasky Medical Center and Maccabi Healthcare Services, is Senior Lecturer in Medicine at the Sackler Faculty of Medicine at Tel Aviv University, and serves on the editorial boards of a number of leading journals in the field of antimicrobial chemotherapy and infection control. His primary research interest is the epidemiology of antimicrobial resistance.